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Polyaniline/MnO_2 nanocomposites based stainless steel electrode modified enzymatic urease biosensor

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Abstract

Polyaniline/MnO_2 nanocomposites in different weight percent (5%, 10% and 15%) were prepared via in-situ chemical oxidation polymerization method. As-synthesized nanocomposites were electrochemically deposited on the stainless steel electrode. These modified electrodes were immobilized with urease by microencapsulation method. During deposition of the PANI/MnO_2 nanocomposite on the stainless steel surface, it undergoes surface oxidation during the initial potentiometric scan of the deposition. The adsorption of the PANI/MnO_2 nanocomposite takes place on the electrode surface followed by the oxidation step of stainless steel electrode. The structure of the prepared composite has been characterized by FTIR and X-ray diffraction (XRD) techniques. Cyclic voltammetry (CV) was carried out in 0.5 M NaOH for pure PANI and PANI/MnO_2 nanocomposites of 5%, 10% and 15% of MnO_2 in the voltage range -200 mV to +1100 mV at the scan rate 50mVs^{-1}. From the cyclic voltammetry study, it can be seen that both the cathodic and anodic currents are higher at higher urease concentration. The storage stability of the PANI/MnO_2/Urease biosensor electrode has also been studied. The adsorption of the urease with PANI/MnO_2 modified steel electrode exhibited good urease biosensor.

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Keywords: Polyaniline, MnO_2, Nanocomposites, Cyclic Voltammetry, Urease Biosensor

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1. Introduction

Due to the high toxicity of heavy metals, it is crucial to detect ultra low levels of the metals, especially in drinking

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water. Heavy metals which affect the surface water systems are cadmium, chromium, mercury, lead, arsenic, and antimony. Various electrochemical biosensors have been reported so far for heavy metal ion detection by immobilizing different enzyme on various transducers (Nomngongo etal, 2011 and Chey et al, 2012). Johnson et al, (2014), reported the approaches and challenges of understanding enzymatic acceleration at nanoparticle interfaces. Soujanya et al, (2014) reported electrospun nanofibrous polymer coated magnesium alloy for biodegradable implant applications. The common techniques include spectrometric techniques such as inductively coupled plasma- atomic emission spectroscopy, ICP-AES (Zhylyak et al, 1995), as well as anodic stripping voltammetry. Even though ICP techniques have low detection limits ranges from parts per billion, ppb to parts per trillion (Renedo et al, 2007), however, they are unsuitable for in-situ analysis, they are expensive, sophisticated and require skilled operators. For these reasons, the development of alternative techniques such as electrochemical biosensor techniques, offer alternative methods because they are sensitive, low cost and simple to operate, (Turdean, 2011). Recent developments have shown the use of electrochemical biosensors as indirect methods for detection of Cd^{2+}, Cu^{2+}, Cr^{3+}, Zn^{2+}, Ni^{2+} and Pb^{2+} using urease biosensor (Narinesingh et al, 1994); Cd^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} and Pb^{2+} using alkaline phosphatise; Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} by glucose oxidase (Volotovsky et al, 1997); Hg^{2+} using glucose oxidase invertase and mutarose (Contractor et al, 1994); Cu^{2+}, Cd^{2+}, Mn^{2+} and Fe^{3+} using acetylcholinesterase (Malhotra et al, 2006); and Cu^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+} by nitrate reductase (Rodriguez et al, 2004). The urease biosensor has so far only been reported for detection of mercury (Gvozdenović et al, 2011and Yano Jun et al, 1997). The recent research in the field of biosensor is limited to electropolymerization on substrate such as Pt, Au and glassy carbon electrodes. However, polymerization can be done on active substrate electrodes such as Cr, Ti, Al, Zn, brass, stainless steel (Adhikari and Majumdar, 2004 and Camalet et al, 1998). Among all these stainless steel is comparatively low cost than other electrodes. Electropolymerized stainless steel electrode then can be used for immobilizing enzyme such as urease in polymeric material matrix (Camalet et al, 2000). The entrapment of enzyme on electrodeposited layer can be improved by using composite of nanostructured metal oxide with polymer material (Ali, 2002) as nanostructure particles provide high surface to volume ratio.

Electrochemical biosensors are based on monitoring electroactive species that are either produced or consumed by the action of the biological components e.g., enzymes and cells (Ran et al, 2010). The determination of heavy metal ions using the urease-immobilized biosensor is based on the measurement of the urease enzymatic activity, which is inhibited by heavy-metal ions. The enzyme urease plays a very important role by catalyzing the decomposition reaction of urea (Dominguez et al, 2009). It has been shown that a properly chosen immobilization method could considerably improve enzyme inhibitor sensitivity compared to that of free enzyme (Malitesta and Guascito, 2005). The presence of heavy metal ion inhibits the enzyme that leads to a decrease in enzymatic activity and, as a result, a lower quantity of ammonium is liberated. Inhibited reaction allows the determination of the presence of metal ions, (Kuralay et al, 2007). We are introducing a novel method of immobilization of urease on a nanostructure MnO_2/PANI composite film on stainless steel by microencapsulation, which has not been used earlier as far as our knowledge.

2. Experimental

2.1. Materials

Aniline, $MnSO_4$, $KMnO_4$ and $(NH_4)_2S_2O_8$ were procured with high purity from Merck Ltd, Mumbai. Urease (Jack bean mill) was procured from Loba Chemie, India. All chemicals were used as received without further purification except aniline monomer which was purified and kept below 4^{o}C. All chemical used were of analytical grade and double distilled water was used for solution preparation.

2.2. Synthesis of PANI-MnO_2 nanocomposites

A mixture of $MnSO_4$ (1.0M) and $KMnO_4$ (0.5M) solution was stirred for 4 h at 70 ◦C. The obtained precipitate was washed several times with double distilled water followed by ethanol to remove impurities and then dried in vacuum at 110 ◦C for 5 h. The dried powder was put into muffle furnace and heated at 300 ◦C for 3 h. This powder was acidified with 2.0M H_2SO_4 at 90 ◦C for 2 h. Finally, the product was washed with distilled water and vacuum dried (Chen Liang et al, 2010). PANI/MnO_2 nanocomposites were chemically synthesized by oxidative

polymerization of aniline using ammonium peroxydisulfate ($(NH_4)_2S_2O_8$) under controlled conditions. As-synthesized MnO_2 in different weight percentage 5%, 10%, 15% and 20% was added to acidic solution of aniline and then ammonium peroxydisulfate was added drop wise for polymerization. The dark precipitate of the PANI/MnO_2 nanocomposites was recovered by filtration and washing several times using double distilled water and methanol for the elimination of the low molecular weight polymer and oligomers. Further, this precipitate was dried at 80 ^oC in vacuum oven.

2.3. Electrochemical deposition of PANI/MnO_2 nanocomposites

The electrodeposition of PANI/MnO_2 nanocomposites was performed using Potentiostat (CH-600D). Platinum wire was used as counter electrode, $Ag/AgCl_2$ as reference electrode and stainless steel as working electrode. The layer of PANI/MnO_2 nanocomposites with different weight percentage were deposited on Stainless Steel surface by linear swiping voltage in range -200 mV to +1100 mV at the scan rate 50 mVs^{-1} for 20 cycles. After 20 cycles the sufficient amount of layer was deposited. The electrodes were washed with double distilled water, dried at room temperature and used for further studies.

2.4. Enzyme Immobilization

The immobilization of urease on PANI/MnO_2 nanocomposites deposited stainless steel electrode was done using a novel microencapsulation method. In a typical process, 1.5 gm of Sodium Alginate was dissolved in 50 ml distilled water. 1.2 gm of Urease was dissolved in 50 ml distilled water, separately. These two solutions were mixed and stirred for 30 minutes. The electrodeposited stainless steel electrode was dipped in this solution for 5 minutes. This electrode further dipped into ice cooled 0.2 M $CaCl_2$ solution. Suddenly, capsules of urease were formed covering the electrode. The electrodes were dipped in a pH=4 acetic acid solution, washed with water and then left overnight at 5^oC in pH =5.6 phosphate buffer solution. The next day, the membrane was washed with pH=7 phosphate buffer solution. The prepared PANI/MnO_2/Urease electrodes were stored below 4^oC.

3. Results and Discussion

3.1. X-ray diffraction

Fig. 1 shows the XRD patterns of PANI and PANI/MnO_2 10%. XRD of pure PANI shows polycrystalline whereas that of PANI-MnO_2 10% shows crystalline. For the as-synthesized PANI-MnO_2, the diffraction peaks at 2θ = 12.7, 18.1, 28.8, 37.4, 49.8 and 60.2 degree can be indexed to a pure tetragonal phase of α-MnO_2 (JCPDS 44-0141). Good crystalline structure is evidenced by the strong diffraction peaks and no other characteristic peaks of impurities are observed in PANI-MnO_2 10%. The grain size of the PANI-MnO_2 10% was determined using Scherer formula: $D=0.9\lambda/\beta\cos\theta$, where, λ is the wavelength of x-rays used, β is the full width at half maximum and θ is the corresponding position. The estimated grain size from most intense peak was found to be 60 nm.

3.2. Fourier Transform Infra Red spectroscopy

Fig. 2 shows the typical FT-IR spectra of PANI and PANI-MnO_2 10%. Both the spectra shows the high wave number bands at 3430 cm^{-1} and 2960 cm^{-1} correspond to N H stretching and aromatic C H stretching respectively. Among the various peaks assigned to PANI, the characteristic peaks around 1564 and 1447 cm^{-1} relate to the stretching vibration of quinoid (−N=(C_6H_4)=N-) ring and benzenoid (−(C_6H_4)-) ring, respectively. Another main band at 1300 cm^{-1} can be assigned to the stretching of C-N in -NH-(C_6H_4)-NH-. The bands appeared at 1103 cm^{-1} and 809 cm^{-1} which correspond to the stretching of C-H in-plane and C-H out-of-plane bending (Nandapure et al, 2013). The band became weaker as shown from PANI-MnO_2 10%, which suggests that the doping degree of PANI is changed with the MnO_2. The characteristic bands of PANI-MnO_2 10% shifted right compared with that of pure PANI, which is ascribed to the effect of MnO_2 on PANI. It demonstrates that some special interaction exists between MnO_2 and PANI.

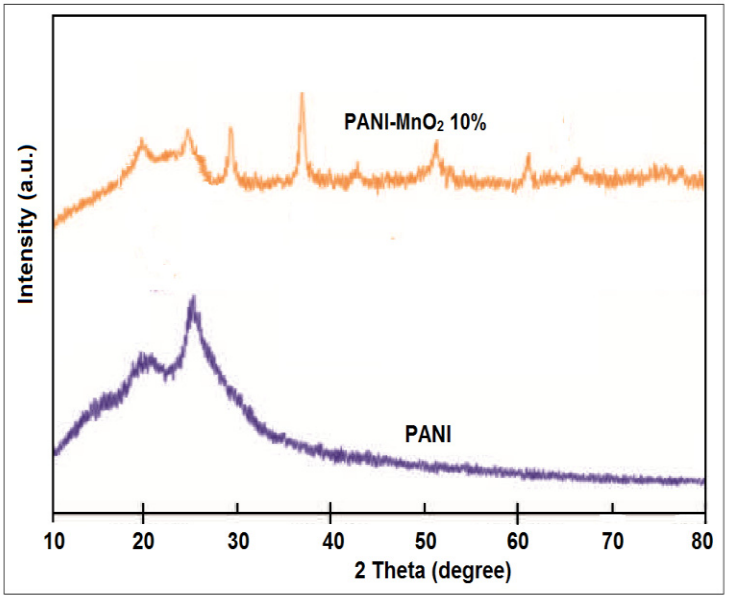

Fig. 1. XRD patterns of PANI and PANI/MnO_2 nanocomposite

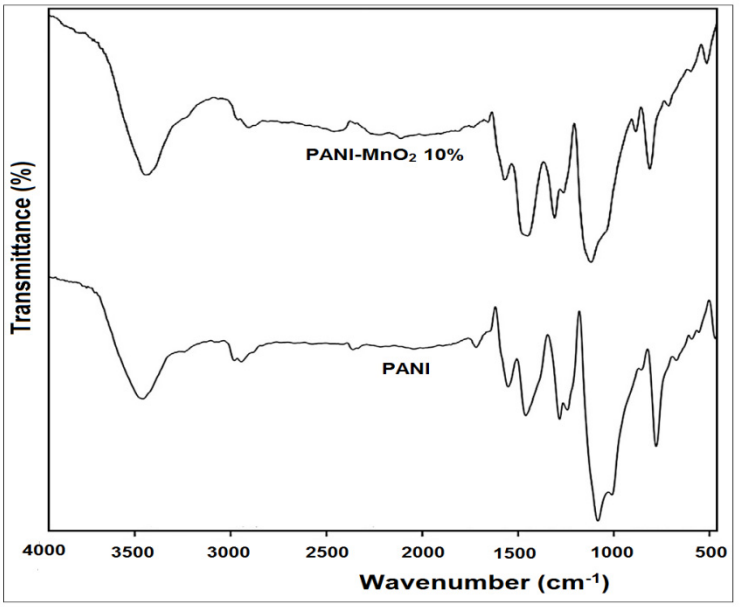

Fig. 2. FTIR spectra of PANI and PANI/MnO_2 nanocomposite

3.3. Cyclic voltammetry study

Electrochemical test was performed in a three electrode cell configuration containing stainless steel (as the working electrode), Pt wire (as the counter) and Ag/AgCl (as the reference). Cyclic voltammetry (CV) was carried out in 0.5 M NaOH for pure PANI and PANI/MnO_2 nanocomposites of 5%, 10% and 15% in the voltage in range -200 mV to +1100 mV at the scan rate 50mVs^{-1}. Fig 3 shows the cyclic voltammetry of PANI/MnO_2 nanocomposites for different concentration of MnO_2, electrodeposited on stainless steel electrode immobilized with urease. The CV show that the potential reversals at the potential limits are fast and instantaneous. This suggests that the adsorption/desorption process of the ions are very fast during cycling (Mahore et al, 2014). The oxidation-reduction current for PANI/MnO_2 nanocomposites was found to be increased with an increase in concentration of MnO_2. From the cyclic voltammetry study, it can be seen that both the cathodic and anodic currents are higher at higher urease concentration. This may be due to the increase in proton concentration in the electrolyte, which favours rapid protonation–deprotonation giving rise to larger currents. Thus, conducting polyaniline having amine functional group can be utilized as a suitable matrix for the physical adsorption of urease.

Chronoamperometric response of the PANI/MnO_2/urease biosensor in the presence of urea was studied. All the measurements were carried out in electrochemical cell, containing 20 ml of 0.1 M PBS (pH = 7.2) with successive addition of 0.1 ml of 10 mM urea at potential of −0.6 V. It was observed that the current increased with the addition of urea which is due to the produced ammonium from the enzymatic reaction, then reached saturation. The urea calibration curve of PANI/MnO_2/urease biosensor is shown in Fig. 4(a). It can be seen that with increasing concentration of urea the current also increased linearly. The greater sensitivity was due to the incorporation of MnO_2 deposited PANI film of the electrode. The storage stability of the PANI/MnO_2/Urease biosensor electrode has also been studied. Fig. 4(b) shows the response of electrodes for 10mM of urea. It can be seen that there is decline in response after about 6 days followed by a gradual decrease. After about 20 days the sensor response is still significant and thus this biosensor can be used for urea determination for about 20 days. This efficient physical adsorption of the urease with PANI-MnO_2 modified steel electrode exhibit a good urease biosensor. This low cost PANI-MnO_2 nanocomposite deposited urease modified stainless steel electrodes can be used as a good transducer for sensing heavy metal ions in water.

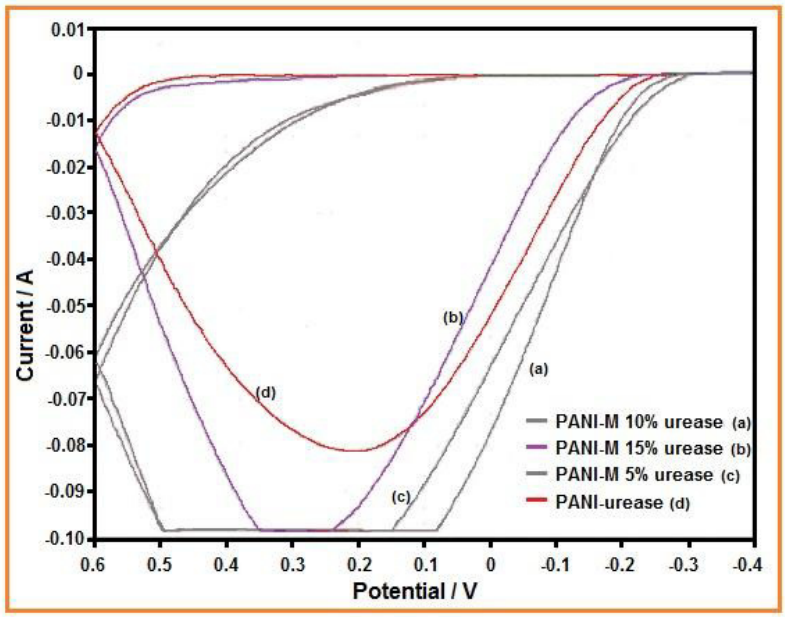

Fig. 3. Cyclic voltametry of PANI/MnO_2/Urease stainless steel electrode: Pure PANI, 5%, 10% and 15% of MnO_2

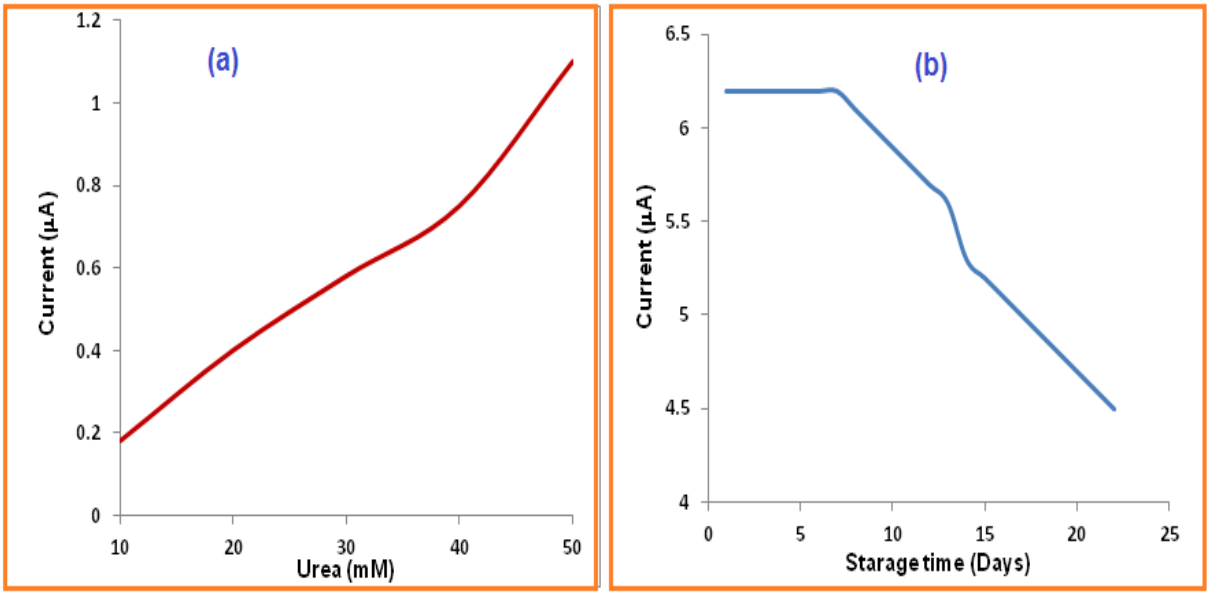

Fig. 4. (a) Urea calibration curve and (b) Storage stabilization of PANI/MnO_2/Urease biosensor

4. Conclusion

PANI/MnO_2 nanocomposites have been successfully synthesized by an in-situ polymerization. Structural characterization shows the interaction of PANI and MnO_2. The urea calibration curve of PANI/MnO_2/urease biosensor shows increase in current linearly with increasing concentration of urea. After about 20 days the sensor response is still significant and can be used for urea determination. This efficient physical adsorption of the urease with PANI-MnO_2 modified steel electrode exhibited good urease biosensor. The experiments are presently in progress to improve the shelf lifetime of this stainless steel PANI/MnO_2/Urease electrode and selectivity towards Hg^{2+}, Pb^{2+}, Cu^{2+} heavy metal ions commonly found in ground water in Nagpur Region of Maharashtra State (India).

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